CHEMISTRY OF OXIDATION OF POLYCYCLIC AROMATIC HYDROCARBONS BY SOIL PSEUDOMONADS

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Received for publication November 16, 1961

ABSTRACT

ROGOFF, MARTIN H. (U.S. Bureau of Mines, Pittsburgh, Pa.). Oxidation of polycyclic aromatic hydrocarbons by soil pseudomonads. J. Bacteriol. **83**:998–1004. 1962.—Substitution of phenanthrene by a methyl group at the 9-carbon blocks oxidation of the compound by a resting-cell suspension of a phenanthrene-grown soil pseudomonad. When 2-methylphenanthrene is provided, the oxidation rate is considerably higher; 3-methylphenanthrene is oxidized at a rate intermediate between the other two, even though the methyl group is attached to a carbon directly involved in ring splitting. Cells grown on naphthalene or anthracene oxidize phenanthrene at a much lower rate than cells grown with phenanthrene or 2-methylnaphthalene as the source of carbon. Naphthalene-grown cells also absorb less phenanthrene from aqueous solution than do their phenanthrene-grown counterparts.

The data are in keeping with the hypothesis that polynuclear aromatic hydrocarbons attach to the relevant bacterial enzymes at carbon-carbon bonds of high electron density (K regions; localized double bonds), and that the ring-splitting reactions then occur at other bonds on the substrate molecule. The actual bond that undergoes fission is determined by the electronic and steric configurations of the enzyme-substrate complex. When linearly arranged aromatic compounds such as naphthalene or anthracene are attacked, attachment to an enzyme and ring splitting may take place on the same ring; angular aromatic compounds such as phenanthrene afford attachment to an enzyme at a bond in a ring other than the one containing the ring-splitting site.

Previous reports on the bacterial degradation of polynuclear aromatic hydrocarbons outlined the general metabolic pathways that these oxidations follow (Rogoff and Wender, 1957*a*,*b*, 1959; Fernley and Evans, 1958; Colla, Biaggi, and Treccani, 1957). Examination of these metabolic pathways reveals that the first ring to undergo cleavage is not a ring bearing a substituent group (CH_3, Cl) and seldom a ring bearing an electron-dense carbon-carbon bond (K region). [Pullman and Pullman (1955) proposed that carcinogenic polynuclear aromatic hydrocarbons have two regions important in determining their chemical behavior. The carbon atoms of these regions have a high electron density, as exemplified by the bond at the 9,10-carbons of phenanthrene (K region) and the region of the 9 and 10 carbons of anthracene (L region).]

The hydroxylation of polynuclear aromatic hydrocarbons in animal tissues usually does not occur at the chemically most reactive bond in the molecule (Pullman and Pullman, 1955). The reactive electron-dense region becomes attached to the tissue, blocking hydroxylation at this site. Other bonds in the hydrocarbon are activated in the additional complex formed between hydrocarbon and tissue (Pullman and Baudet, 1954); hydroxylation takes place at these newly formed sites of secondary reactivity (Bhargava and Heidelberger, 1956; Bhargava, Hadler, and Heidelberger, 1955). Bhargava and Heidelberger (1956) demonstrated that the K region (3,4-bond) of 1,2:5,6-dibenzanthracene is involved in linkage with animal tissues.

The first reactions in the oxidation of polynuclear aromatic hydrocarbons lead to the formation of dihydroarenediols, both in animal tissues (Parke and Williams, 1959) and under bacterial attack (Colla, Biaggi, and Treccani, 1959; Fernley and Evans, 1958). The positions of hydroxylation are often the same in both cases.

Because similarities exist between the mode of attack on polynuclear aromatic hydrocarbons by bacterial and animal enzymes, it seems reasonable that electron-dense regions are active as regards

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the bacterial oxidizing systems. Polynuclear aromatic hydrocarbons may attach to bacterial enzymes through these reactive sites, thus giving these sites some directive influence on the subsequent course of bacterial oxidation. Adoption of such an attachment as a working hypothesis might help to explain certain observations made during studies of the oxidation of various polynuclear aromatic hydrocarbons by soil pseudomonads.

The experiments described in this paper include studies on (i) cross-adaptation to oxidation of various polynuclear aromatic hydrocarbons, (ii) selective absorption of these compounds by induced bacterial cells, and (iii) oxidation rates observed when cells grown on certain polynuclear hydrocarbons are allowed to oxidize substituted derivatives of these compounds.

MATERIALS AND METHODS

Media. A mineral-salts medium was prepared as two separate salt solutions. Solution A contained (g/500 ml): NH_4NO_3, 2.5; $MgSO_4 \cdot 7H_2O$, 0.5; $MnCl_2 \cdot 4H_2O$, 0.1; $FeCl_2 \cdot 4H_2O$, 0.05; $CaCl_2$, 0.01; $CaCO_3$, 2.0. Solution B contained (g/500 ml): Na_2HPO_4, 1.0; KH_2PO_4, 0.5. The salt solutions were sterilized separately, and equal volumes were mixed after cooling. When a solid medium was required, 2.0% agar was added to the complete, filtered medium before sterilization. Yeast extract (or Bacto-Casitone), when used as a supplement, was added at a concentration of 0.05% to solution B before sterilization. Sterile polynuclear aromatic hydrocarbons (sources of carbon) were ground aseptically and added to the media at a 1.0% concentration.

Cultures. The soil pseudomonads closely resembling *Pseudomonas aeruginosa* used in these experiments have been described previously (Rogoff and Wender, 1957*a*,*b*). The cultures were maintained in mineral-salts broth with an appropriate polynuclear hydrocarbon as the source of carbon. Periodic plating was carried out to maintain purity of the cultures. Cells were considered induced to utilize a particular hydrocarbon after a minimum of three transfers on mineral-salts agar plates with the inducing hydrocarbon as sole source of carbon. Although the cultures could be readily transferred from one source of carbon to another, at least two transfers were required to initiate rapid growth.

Washed-cell suspensions. When naphthalene or 2-methylnaphthalene was used as the source of carbon, cell crops were grown in pans (6 by 10 in.) containing mineral-salts agar (200 ml/pan); the pans were inverted over crystals of the compounds placed on aluminum foil. When phenanthrene was the source of carbon, the cells were usually grown in 1-liter portions of liquid medium in 4-liter Erlenmeyer flasks. These were incubated on a reciprocating shaker (180 rev/min, 2-in. stroke). Phenanthrene-grown cells were also cultured with the compound, sprinkled directly onto the surface of agar media. Samples of broth cultures grown with the appropriate polynuclear aromatic hydrocarbons as source of carbon served as inocula.

After 18 to 48 hr of incubation on agar at room temperature, the cells were washed from the agar with 0.05 M phosphate buffer (pH 7.2), and filtered through glass wool; liquid cultures were filtered directly. The cells were removed from the filtrate by centrifugation at 5 C, washed twice, and resuspended in buffer. Cell suspensions were adjusted to contain 1.5 to 2.0 mg bacterial N per ml. Exact bacterial N content was determined by the Kjeldahl method. Cell crops could be stored in the refrigerator for 2 to 4 weeks, although lowered $Q_{O_2}(N)$ values were obtained with cells stored for this period

Manometric methods. Oxygen uptake by cell suspensions was measured by use of Warburg manometers in the conventional manner (Umbreit, Burris, and Stauffer, 1957). Polynuclear aromatic hydrocarbon substrates were prepared as stock acetone solutions containing 20 μmoles per ml. Suitable samples of the stock solutions were placed in the main flask compartment, and solvent was removed by warming under reduced pressure. Each flask contained 0.5 ml of cell suspension in the side arm, 0.15 ml of 20% KOH, and a 2-cm square of Whatman no. 40 filter paper in the center well, and 10 μmoles of substrate plus 2.3 ml of phosphate buffer (0.05 M, pH 7.0) in the main compartment. The total volume of the flask contents was 3.0 ml. The temperature was 30 C; the gas phase was air.

Absorption spectra. Ultraviolet absorption by polynuclear aromatic hydrocarbons in aqueous solution was measured in a Carey model 11 recording ultraviolet spectrophotometer. Stock solutions of the appropriate hydrocarbons were prepared by suspending finely ground crystals in phosphate buffer (0.02 M, pH 7.38). The sus-

pensions were held at 37 C for 72 hr, and then were filtered through Whatman no. 41H filter paper. The filtrate was used for determining the ultraviolet spectrum and in the absorption experiments. Infrared analyses of the compounds prepared in potassium bromide pellets were made, using a Perkin-Elmer model 21 double-beam infrared spectrophotometer.

Chemical compounds. Polynuclear aromatic hydrocarbons were tested for purity by comparison of their infrared spectra with samples of known purity. If not sufficiently pure, the hydrocarbons were recrystallized from appropriate solvents and rechecked spectrophotometrically.

RESULTS

Manometric experiments with substituted phenanthrenes. An indication that electron-dense regions might play a role in the bacterial oxidation of polynuclear aromatic hydrocarbons was obtained when oxidation rates of the parent compounds and some substituted derivatives were compared. Suspensions of phenanthrene-grown cells were placed in the Warburg vessel with phenanthrene, 9-methylphenanthrene, or 9-ethoxyphenanthrene as substrate. The data obtained (Fig. 1) show that substitution at the 9-position lowers the availability of the phenanthrene nucleus to the organism. The effect was not due solely to the lower solubility of the substituted phenanthrenes. The ultraviolet spectra of the compounds show that phenanthrene is only 2.4 times more soluble in water than 9-methylphenanthrene, yet in this instance is oxidized at almost 6 times the rate of the substituted compound. Solubility of 9-ethoxyphenanthrene lies between those of the two hydrocarbons. Attempts to use 9-methylphenanthrene as a source of carbon for growth of the organism were unsuccessful.

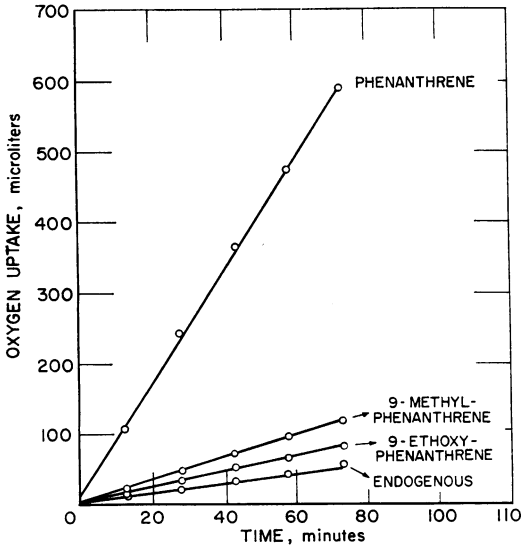

FIG. 1. *Oxygen uptake by a phenanthrene-grown soil pseudomonad in the presence of phenanthrenes substituted at the 9-position. The Warburg vessel contained 1.0 mg bacterial N and 10 μmoles of substrate in 0.05 M phosphate buffer (pH 7.0); the final volume was 3.0 ml.*

The 9,10-bond of phenanthrene, although most reactive in chemical addition reactions, is not the site of initial ring splitting during bacterial oxidation (Rogoff and Wender, 1957*a*); the 3,4-bond is the first carbon-carbon bond cleaved (Colla et al., 1959). The data in Fig. 1 clearly indicate involvement of the 9,10-bond in the bacterial attack on the molecule, although not at a ring-splitting site. To determine the effects of methyl substitution at other bonds in the molecule, the rates of oxidation of phenanthrene substituted at the 2-, 3-, or 9-position were determined manometrically in the presence of suspensions of resting cells grown on phenanthrene, naphthalene, or 2-methylnaphthalene (Table 1). It was found that the oxidation rates of cells oxidizing the phenanthrene series followed the order: phenanthrene > 2-methylphenanthrene >

TABLE 1. *Respiration of soil pseudomonads grown on different polynuclear aromatic compounds in the presence of phenanthrene and some monomethyl derivatives**

Substrate	$Q_{O_2}(N)$† of cells grown on		
	Naphthalene	Phenanthrene	2-Methylnaphthalene
Phenanthrene	172	716	472
2-Methylphenanthrene	128	396	230
3-Methylphenanthrene	74	210	188
9-Methylphenanthrene	28	78	42

* Oxygen uptake was determined in Warburg manometers with 10 μmoles of substrate provided. Endogenous respiration was subtracted before calculation.

† $Q_{O_2}(N)$ represents μliters of O_2 taken up per mg cell N per hr. Q_{O_2} values for freshly harvested cells were considerably higher than those for cells stored over extended periods at 5 C; however, the relative oxidation rates were retained regardless of age of the cells.

3-methylphenanthrene > 9-methylphenanthrene, regardless of which hydrocarbon was used as the growth substrate.

Substitution at the reactive 9,10-bond of phenanthrene is more effective than blocking at the 3-carbon, one of two possible sites of ring splitting (the 3,4- and 5,6-bond are equivalent). Because only one of two attackable sites is blocked in 3-methylphenanthrene, oxidation proceeds but at a significantly lower rate than was found for 2-methylphenanthrene, where the blocked position is not at the ring-splitting site. The initial oxidation rate of 2-methlyphenanthrene is not maintained over a period of time but falls off during the time in which the rate of phenanthrene oxidation remains constant. This may be owing to steric hindrance, by the methyl group, of enzymatic manipulation of a methyl-substituted intermediate formed as a result of ring splitting.

Cross-induction experiments. Phenanthrene was oxidized at a lower rate, in relation to oxidation of the methyl-substituted compounds by the naphthalene-grown cells, than in the case of the phenanthrene- or 2-methylnaphthalene-grown cells. This suggested a possible difference in the mode of attack by these bacteria on naphthalene as opposed to phenanthrene. An experiment was designed to determine rates at which different polycyclic aromatic hydrocarbons were oxidized by cells grown with the various polycyclics as sources of carbon. Cells of the pseudomonad were grown with naphthalene, 2-methylnaphthalene, anthracene, or phenanthrene as source of carbon. The rates of oxygen uptake by the harvested cells were measured in a Warburg apparatus in the presence of naphthalene, 2-methylnaphthalene, or phenanthrene. Cells grown on any of the four polynuclear aromatic hydrocarbons oxidize the other compounds when supplied as substrates (Table 2). This occurred with no demonstrable lag period. The results also indidate that phenanthrene- or 2-methylnaphthalene-grown cells oxidize naphthalene and 2-methylnaphthalene at equivalent rates; phenanthrene is oxidized at about one-third that rate. Cells grown on naphthalene or anthracene, however, show lower rates for the oxidation of phenanthrene and 2-methylnaphthalene than cells induced with 2-methylnaphthalene or phenanthrene.

The preceding data demonstrate that in phenanthrene oxidation the 9,10-bond is involved,

TABLE 2. *Respiration of an aromatic hydrocarbon-attacking pseudomonad, grown on different polynuclear aromatic hydrocarbons, in the presence of certain growth compounds*

Cells grown on	Substrate	$Q_{O_2}(N)$*	Relative oxidation rate (naphthalene = 1.00)
Naphthalene	Naphthalene	612	1.00
	2-Methylnaphthalene	456	0.73
	Phenanthrene	69	0.11
Anthracene	Naphthalene	305	1.00
	2-Methylnaphthalene	157	0.52
	Phenanthrene	37	0.12
Phenanthrene	Naphthalene	374	1.00
	2-Methylnaphthalene	359	0.96
	Phenanthrene	110	0.30
2-Methylnaphthalene	Naphthalene	639	1.00
	2-Methylnaphthalene	618	0.97
	Phenanthrene	359	0.39

* $Q_{O_2}(N)$ represents μliters of O_2 taken up per mg cell N per hr. Oxygen uptake was measured in Warburg manometers at 30 C with 10 μmoles of substrate provided to 0.5 to 1.0 mg cell N.

even though it is not a ring-splitting site, and that there are differences in the enzymes elaborated by these bacteria in response to naphthalene as opposed to phenanthrene. These differences are reflected in the rates at which appropriately induced cells oxidize the test compounds. If we assume that the 9,10-bond of phenanthrene is involved as the site of attachment to the relevant enzyme through an addition reaction, then it is apparent from the structural formula of the phenanthrene molecule (Fig. 2) that there are no unsubstituted carbons on the center ring that would serve as sites for enzymatic hydroxylation. Ring cleavage occurs between the 3- and 4-carbons (Colla et al., 1959), and the enzyme, if coupled to the molecule at the 9,10-bond, must span the diagonal distance across the molecule to the 3,4-bond. Attachment of naphthalene to an enzyme is most likely to take place at the 1,2-bond (the 3,4-, 5,6-, or 7,8-bonds are all equivalent to the 1,2-bond), according to Pullman (1954), and not at the 2,3-bond (Efros, 1960). An attachment at the 1,2-bond of naphthalene would leave the 3,4-bond on the same ring as an available oxidative site, with a much shorter intramolecular distance than in the case of phenanthrene for the span between the involved sites.

The rates of oxidation of some dimethylnaph-

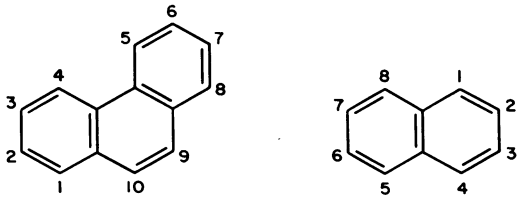

FIG. 2. *Structure of phenanthrene and naphthalene.*

thalenes by appropriately induced cells tend to substantiate this approach as a possible explanation of the differences observed in the induced enzyme patterns of naphthalene-grown as opposed to phenanthrene-grown cells. The rates at which naphthalene-, phenanthrene-, and 2-methylnaphthalene-grown cells oxidized naphthalene and 2,3- and 2,7-dimethylnaphthalene were determined manometrically. Phenanthrene- and 2-methylnaphthalene-grown cells oxidize 2,7-dimethylnaphthalene at an even higher rate than they do the 2,3-isomer; the reverse order is observed when naphthalene-grown cells are allowed to oxidize the test compounds (Table 3). The very low rate of oxidation observed with naphthalene-grown cells on 2,7-dimethylnaphthalene, in which both rings of the compound are blocked by methyl groups, supports the idea that, in naphthalene attack, oxidation and attachment to an enzyme occur in the same ring.

Absorption of polynuclear aromatics from aqueous solution by suspensions of resting cells. Another expression of the aforementioned effects was found when the absorption of polynuclear aromatic hydrocarbons from aqueous solution by hydrocarbon-induced cells was measured. Because the molecular size of the test compounds would probably prevent their entry into the cell, their oxidation might occur on the cell wall; the absorptive capacity of the cell wall, therefore, might reflect the activities of the enzymes it contained. Ultraviolet spectra of samples of polynuclear aromatic compounds were determined, and their solubilities were calculated. The procedure used to determine absorption of the hydrocarbons from solution by suspensions of resting cells follows. Samples (40 ml) of the filtered hydrocarbon solutions were placed in 75-ml Lusteroid centrifuge tubes. Cell suspensions were adjusted to contain 1.0 mg bacterial N per ml, and 1-ml samples of various dilutions were added to the tubes; a blank tube received 1.0 ml of buffer. The tubes were allowed to stand for 3 min and were then centrifuged at 3,500 $\times$ *g* for 20 min. The supernatant fluids were decanted; samples were placed in the ultraviolet cuvettes; and the spectra were recorded. A 10-cm quartz cell was used when phenanthrene absorption was measured; the 1-cm cell proved adequate for the more soluble naphthalene. Concentrations of the hydrocarbons were calculated from the spectra by the base line method (Wright, 1941). Data obtained from several experiments, summarized in Table 4, represent the average values obtained from at least two dilutions of the same cell suspension. The naphthalene-grown cells do not absorb phenanthrene from solution to the same extent as do the phenanthrene-grown suspensions, although the amount of naphthalene absorbed by both does not differ significantly.

TABLE 3. *Respiration of an aromatic hydrocarbon-attacking pseudomonad, grown on different polynuclear aromatic hydrocarbons, in the presence of naphthalene and certain dimethylnaphthalenes*

Cells grown on	Substrate	$Q_{O_2}(N)$*	Relative oxidation rate (naphthalene = 1.00)
Naphthalene	Naphthalene	720	1.00
	2,3-Dimethylnaphthalene	214	0.30
	2,7-Dimethylnaphthalene	67	0.09
Phenanthrene	Naphthalene	956	1.00
	2,3-Dimethylnaphthalene	310	0.32
	2,7-Dimethylnaphthalene	464	0.49
2-Methylnaphthalene	Naphthalene	742	1.00
	2,3-Dimethylnaphthalene	312	0.42
	2,7-Dimethylnaphthalene	378	0.51

* Q_{O_2} (N) represents μliters of O_2 taken up per mg cell N per hr. Oxygen uptake was measured in Warburg manometers at 30 C with 10 μmoles of substrate provided to 0.05 to 1.0 mg cell N.

TABLE 4. *Absorption of naphthalene and phenanthrene from aqueous solution by resting cells of a soil pseudomonad grown with one of the compounds as a source of carbon*

Cells grown on	Hydrocarbon	Solubility*	Hydrocarbon absorbed/mg bacterial N		Absorption (mole ratio) naphthalene/phenanthrene
		mg/liter	*mg*	*moles* $\times 10^{-6}$	
Naphthalene	Naphthalene	37.0	0.279	2.18	5.0
	Phenanthrene	1.5	0.078	0.44	
Phenanthrene	Naphthalene		0.388	3.03	1.1
	Phenanthrene		0.485	2.72	

*Naphthalene measured at 278 mμ, phenanthrene at 252 mμ.

DISCUSSION

From these data, the most salient observation is that the enzymes induced in the pseudomonad by growth with naphthalene as the source of carbon differ from those induced when phenanthrene is provided as a growth substrate. Cells grown on 2-methylnaphthalene display induced enzyme patterns closely resembling the phenanthrene-grown cells; those grown on anthracene show a pattern similar to the naphthalene-grown organism, as regards oxidation of the growth compounds.

Any attempt at explanation of the observed phenomena must be based on an understanding of the chemical characteristics of the molecular structures undergoing oxidation by these bacteria. Not all the bonds in polynuclear aromatic compounds are equivalent. For example, many observations indicate great differences in the properties of the 1,2-bond as opposed to the 2,3-bond of naphthalene (Efros, 1960). In addition reactions, with which we are concerned here, the start of the process of addition to an aromatic bond seems to be its conversion into a localized double bond. In the process, two π electrons, of the general π-electron system of the compounds, are in a sense fixed at the localization site; the remaining electrons may regroup to form a minimum-energy system. The energy necessary to localize double bonds in a number of aromatic compounds has been calculated by the molecular-orbital method (Efros, 1960). The localization energy for the 1,2-bond in naphthalene has been calculated to be 22 kcal, and 37 kcal has been found for the 2,3-bond; that for the 9,10-bond in phenanthrene is only 16 kcal. A value of 30 kcal has been calculated for a bond in benzene. The low localization energies for the 1,2-bond in naphthalene and the 9,10-bond in phenanthrene explain the reactivity of those bonds in addition reactions. The bond in an aromatic molecule having the lowest localization energy requirement may be considered the bond that Pullman and Pullman (1955) call the K region.

The participation of the 9,10-bond of phenanthrene in oxidation by our culture, and the recognized affinity of this bond as the site of addition reactions, lead us to the postulate of attachment of an enzyme to the phenanthrene molecule by means of an addition reaction at the 9- and 10-carbons. Addition of an enzyme should occur in the case of naphthalene and anthracene at a 1,2-bond. With 2-methylnaphthalene, the presence of the methyl group on one of the K-region carbons should increase the electron density at that bond, but should also introduce steric effects on addition reactions at that site (Pullman, 1954). It is therefore not certain at which bond addition of an enzyme might occur in this case, but it is apparent that the induced enzyme patterns observed for 2-methylnaphthalene-grown cells are quite similar to those for their phenanthrene-grown counterparts.

An aromatic compound with electronic characteristics that direct addition of an enzyme at a particular bond imposes on that enzyme a limitation as to which other bonds in the molecule will act as a site for its oxidative (or other) function. This specificity may be due to activation in the enzyme-substrate complex of a bond of normally secondary reactivity in the substrate molecule (Pullman and Baudet, 1954) or may be imposed sterically owing to the configurations of the molecules involved. The question of the site of oxidative or other enzyme function after directed addition of the substrate molecule is then posed. Oxidation of phenanthrene is initiated at the 3,4-bond (Colla et al., 1959), and the enzyme formed in response to this compound must bear a configuration capable of spanning the intramolecular

distance between that bond and the proposed 9,10-bond attachment site. The two-ring span of this enzyme is supported by the observation that 2,7-dimethylnaphthalene is oxidized more readily by phenanthrene-grown cells than is 2,3-dimethylnaphthalene; i.e., an open site on each of two adjacent rings allows more rapid oxidation than two unblocked bonds on one ring.

The enzyme formed in response to naphthalene differs from that formed in response to phenanthrene, as evidenced by the differences in the relative oxidation rates on the substrates tested. Naphthalene-grown cells also readily oxidize 2,3-dimethylnaphthalene in which one ring is completely blocked and the other completely unblocked; they have a low ability to absorb phenanthrene from solution. These observations infer that the addition and oxidation functions of the naphthalene-induced enzyme are spatially closer than those of the phenanthrene-induced enzyme. Further, phenanthrene-grown cells act towards 2,3-dimethylnaphthalene as if they contained a naphthalene-type enzyme, and absorb naphthalene from solution with almost equal efficiency as they do phenanthrene. This is expected since some intermediary metabolites of phenanthrene oxidation are naphthalene derivatives, and a naphthalene-type enzyme would be formed in response to them. The enzyme formed in response to naphthalene then apparently cannot handle a two-ring span, but is capable of oxidizing compounds containing unblocked adjacent double bonds. It is therefore proposed that, in the case of naphthalene, assumed addition to an enzyme and oxidation occur on the same ring.

Growth on anthracene results in induced enzyme patterns resembling those of naphthalene-grown cells; anthracene, like naphthalene, has its K region at the 1,2-bond. On the basis of present data it is difficult to assess whether the methyl groups more profoundly affect enyzme addition or the oxidative functions, but the responses of 2-methylnaphthalene-grown cells closely resemble those grown on phenanthrene; an addition site on one ring with oxidation on the other is therefore inferred in this case.

LITERATURE CITED

BHARGAVA, P. M., H. T. HADLER, AND C. HEIDELBERGER. 1955. The structure of the skin protein-bound compounds following topical application of 1,2,5,6-dibenzanthracene-9, 10-C^{14}. I. 2-Phenylphenanthrene-3,2-dicarboxylic acid, a degradation product. J. Am. Chem. Soc. **77**:2877–2886.

BHARGAVA, P. M., AND C. HEIDELBERGER. 1956. The structure of the skin protein-bound compounds following topical application of 1,2,5,-6-dibenzanthracene-9,10-C^{14}. II. Nature of the 2-phenylphenanthrene-3,2′-dicarboxylic acid-protein bond. J. Am. Chem. Soc. **78**:3671–3677.

COLLA, C., C. BIAGGI, AND V TRECCANI. 1957. Richerche sul metabolismo ossidativo microbico dell'antracene e fenantrene. I: Formazione di acidi ossinaftoici. Atti accad. naz. Lincei, Rend. Classe sci. fis. mat. e nat. **23**:66–69.

COLLA, C., C. BIAGGI, AND V. TRECCANI. 1959. Richerche sul metabolismo ossidativo microbico dell'antracene e fenantrene. II: Isolamento e carraterizzazione del 3,4-diidro-3,4-diossifenantrene. Ann. microbiol. ed enzimol. **9**:1–5.

EFROS, L. S. 1960. The aromatic bond and some problems of the structure of aromatic compounds. Russ. Chem. Rev. **29**:66–78.

FERNLEY, H. N., AND W. C. EVANS. 1958. The oxidative metabolism of polycyclic hydrocarbons by soil pseudomonads. Nature **182**: 673–675.

PARKE, D. V., AND R. I. W. WILLIAMS. 1959. The hydroxylation of foreign aromatic compounds in the animal body. Ann. Rept. Progr. Chem. (Chem. Soc., London) **55**:376–388.

PULLMAN, A. 1954. Electronic structure and carcinogenic activity of aromatic hydrocarbons. Bull. soc. chim. France, p. 595–603.

PULLMAN, A., AND B. PULLMAN. 1954. Electronic structure and carcinogenic activity of aromatic molecules and new developments. Acta Unio Intern. contra Cancrum **10**:150–154.

PULLMAN, A., AND B. PULLMAN. 1955. Cancérisation par les substances chimiques et structure moleculaire. Masson et Cie, Paris.

PULLMAN, B., AND J. BAUDET. 1954. Metabolism of carcinogenic hydrocarbons. Compt. rend. **238**:964–966.

ROGOFF, M. H., AND I. WENDER. 1957*a*. Microbiology of coal. I. Bacterial oxidation of phenanthrene. J. Bacteriol. **73**:264–268.

ROGOFF, M. H., AND I. WENDER. 1957*b*. 3-Hydroxy-2-naphthoic acid as an intermediate in bacterial dissimilation of anthracene. J. Bacteriol. **74**:108–109.

ROGOFF, M. H., AND I. WENDER. 1959. Methylnaphthalene oxidations by pseudomonads. J. Bacteriol. **77**:783–788.

WRIGHT, N. 1941. Application of infrared spectroscopy to industrial research. Ind. Eng. Chem., Anal. ed. **13**:1.